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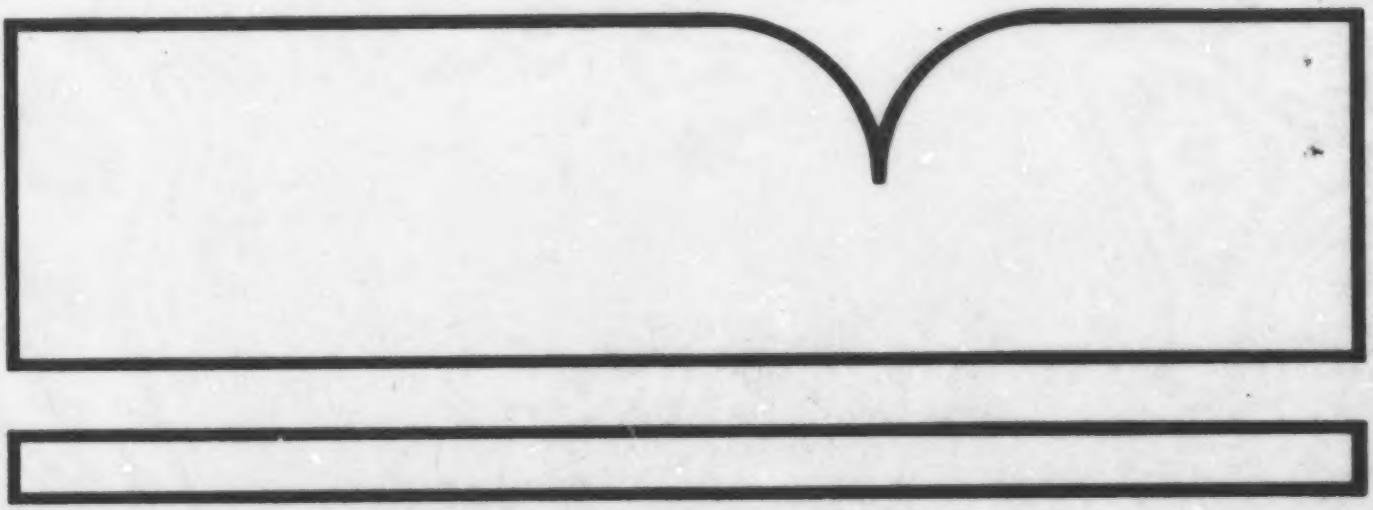
Recommended Guideline for Acute Oral Toxicity Testing in Rodents

(U.S.) Interagency Regulatory Liaison Group
Washington, DC

Jan 81



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TESTING STANDARDS AND GUIDELINES WORK GROUP

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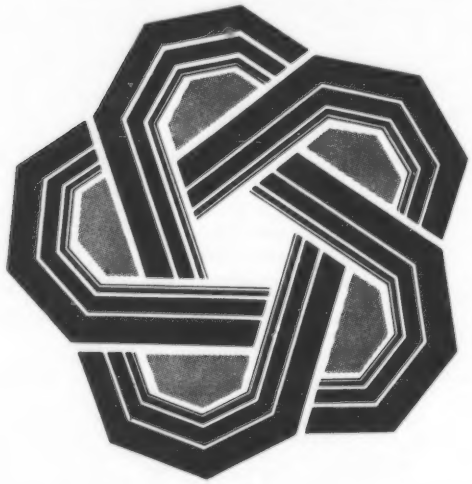

Recommended Guideline For

ACUTE ORAL TOXICITY TESTING IN RODENTS

U.S. Consumer Product Safety Commission
U.S. Environmental Protection Agency
Food and Drug Administration (U.S. Dept. of HHS)
Food Safety and Quality Service (U.S. Dept. of Agriculture)
Occupational Safety and Health Administration (U.S. Dept. of Labor)

BIBLIOGRAPHIC INFORMATION

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PERFORMER: Interagency Regulatory Liaison Group, Washington, DC.

This guideline is designed for use in acute ingestion tests using rodents, but is adaptable to other species. Although several accepted methods for determining LD50 values have been developed, many important determinants of toxicity are not represented either by these values or slopes of dose-response curves for lethality. These determinants are integral to an evaluation of acute toxicity and should be observed during the course of an acute toxicity study. Site and mechanism of action, early or delayed death, and recovery rate may be better indices of toxicity and hazard than LD50 values per se. Morbidity and/or pathogenesis may have more toxicological significance than mortality.

KEYWORDS: *Toxicology, *Toxic substances.

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FOREWORD

Four regulatory agencies, the Consumer Product Safety Commission (**CPSC**), the Environmental Protection Agency (**EPA**), the Food and Drug Administration (**FDA**), and the Occupational Safety and Health Administration (**OSHA**), agreed to work together to reform the regulatory process and to improve protection of workers, public health, and the environment (**42 FR 54856, 11 October 1977**). They formed the Interagency Regulatory Liaison Group (**IRLG**) to implement their agreement. In January 1979, the Food Safety and Quality Service (**FSQS**), Department of Agriculture, joined the IRLG.

These agencies recognized that although they often regulate the same chemicals, toxicity testing guidelines used by each agency are not always uniform. Among currently required tests, differences exist primarily in details of methodology and not in fundamental toxicological principles. The Testing Standards and Guidelines Work Group was established for the purpose of developing guidelines which would resolve existing differences and be used by all of the IRLG agencies for testing chemicals for health or environmental effects or both. This effort was to be coordinated with the development of other guidelines. On December 17, 1977, the Work Group held a public meeting to explain its purpose and goals, and to answer questions about its activities (**42 FR 59108, 15 November 1977**).

IRLG guidelines are derived from those currently used or under development in the agencies, in international organizations, and in industry. Early drafts were circulated through each agency for review, and comments from that review are included in this guideline. In addition, drafts of the EPA Office of Pesticides Programs (**OPP**) proposed guidelines (**43 FR 37336, 22 August 1978**) under the Federal Insecticide, Fungicide and Rodenticide Act (**FIFRA**), and public comments to those guidelines were used extensively in an effort to assure compatibility and to benefit from the appreciable time and effort the OPP staff put into their development. A second major source of information was *Principles and Procedures for Evaluating the Toxicity of Household Substances* (**NAS Pub. No. 1138**), prepared for CPSC by the National Academy of Sciences. Still others heavily relied on were the Pharmaceutical Manufacturers' Association *Guidelines for the Assessment of Drug and Medical Device Safety in Animals* (**February 1977**); the FDA, Bureau of Foods Direct Additive Cyclic Review Draft Guidelines; the *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*, Association of Food and Drug Officials of the U. S.; and protocols submitted by several industrial organizations in response to **43 FR 1987, 13 January 78**. The Work Group also used written and verbal comments received from the public in response to **44 FR 49015, 21 August 1979**. IRLG guidelines are being coordinated with development of guidelines by the Organization for Economic Cooperation and Development (**OECD**). The Work Group acknowledges the contributions from each of these information sources and takes this opportunity to express its appreciation to the scientists who developed them and made them available.

Under most circumstances, the data obtained from this test should be sufficient to meet the requirements for a specific aspect of the toxicological characterization of the test substance. Under certain circumstances, however, the data may show a need for further study; or it may be recognized at the outset this guideline may have to be modified by an agency. It is important that tests be agreed upon between the investigator and the agency requiring them.

Each agency has the responsibility to decide what substance, and what form of the substance, it requires to be tested; which tests it will require; and how it will use the data derived from the tests.

INTRODUCTION

This guideline is designed for use in acute ingestion tests using rodents, but is adaptable to other species.

Although several accepted methods for determining LD_{50} values have been developed, many important determinants of toxicity are not represented either by these values or slopes of dose-response curves for lethality. These determinants are integral to an evaluation of acute toxicity and should be observed during the course of an acute toxicity study. Site and mechanism of action, early or delayed death, and recovery rate may be better indices of toxicity and hazard than LD_{50} values *per se*. Morbidity and/or pathogenesis may have more toxicological significance than mortality.

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I. General Considerations

A. Good Laboratory Practices

Studies should be conducted according to good laboratory practice regulations (e.g., "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," *43 FR 59986, 22 December 1978).*

B. Test Substance

1. The specific substance or mixture of substances to be tested should be determined in consultation with the responsible agency. As far as is practical, composition of the test substance should be known. Information should include the name and quantities of all major components, known contaminants and impurities, and the percentage of unidentifiable materials to account for 100% of the test substance.

2. Ideally, the lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

C. Animals

1. Recommendations contained in DHEW pub. no. (NIH) 74-23, entitled *Guide for the Care and Use of Laboratory Animals*, should be followed for the care, maintenance, and housing of animals.

2. Healthy animals, not subjected to any previous experimental procedures, must be used.

3. The test animal shall be characterized as to species, strain, sex, weight and/or age. Each animal must be assigned an appropriate identification number.

4. Animals may be group-caged for this test unless the pharmacological action of the test substance dictates otherwise, but the number of animals per cage should not prevent continued and clear observation of each animal. When signs of morbidity or excitability are observed in group-caged animals during the test, such animals should be moved to separate cages.

5. Animals should be assigned to groups in a manner that minimizes bias and assures comparability of pertinent variables (for example, weight variation in animals used in a test should not exceed ± 20% of the mean weight).

D. Dead Animals, Necropsy, and Histopathology

Animals should be observed as necessary to insure that there is minimal loss due to cannibalism or similar management problems. Where possible, necropsy must be performed soon after an animal is sacrificed or found dead to minimize loss of tissues due to autolysis. When necropsy cannot be performed immediately, the animal must be refrigerated at temperatures low enough to minimize autolysis and not cause freezer burn. If histopathological examination is to be conducted, tissue specimens should be placed in appropriate fixative when they are taken from the animal.

II. Specific Considerations

A. Test Preparation

1. Animals: Althouth several mammalian test species may be used for the test, the rat is preferred. When attempting to estimate hazards to young humans, additional studies designed to consider the developmental stage of the test animal in relation to anticipated human exposure should be performed.

2. Fasting: Prior to administration of test substance, food should be withheld from rats overnight. For other rodents with higher metabolic rates a shorter period of fasting is appropriate.

3. Limit test: A trial test is recommended to establish the need for further testing. If a test at a dose of at least 5 g/kg body weight using the procedures described for this study produces no compound-related mortality, then a full study using 3 dose levels may not be necessary.

4. Number and sex: At least 10 animals, 5 per sex, should be used at each dose level. Nonpregnant, nulliparous females should be used.

5. Dose levels: At least three and preferably four dose levels should be used to produce a range of toxic effects and mortality rates (*e.g.*, a range from 10 to 90% and bracketing the expected LD_{50}). The data should be sufficient to produce a dose response curve and permit an acceptable determination of the LD_{50}.

6. Controls: Controls are generally not required, since dose response during an LD_{50} may serve as an internal control. If a vehicle or solvent of uncharacterized toxic potential is used, an acute oral toxicity test should be done using the solvent.

B. Test Procedure

1. Route of administration: Ideally, the dose should be administered in a single dose by gavage or capsule. Because of the physical/chemical nature of the test substance, doses may be administered in a suspension or capsules in divided doses over a period of 24 hours.

2. Dosage: The dose is administered via soft rubber or polyethylene tubing or a ball-tip needle. The maximum volume of aqueous solution that can be given in one dose depends on the rodent's size and should not exceed 2 ml/100g body weight. For nonaqueous liquids and suspensions, the volume should not exceed 1 ml/100g body weight. When possible, variability in test volume should be minimized, with concentrations being adjusted accordingly.

3. Observation period: The observation period should be at least 14 days. Although a 14-day observation period is sufficient for most compounds, animals demonstrating visible signs of toxicity after 14 days may be held longer.

4. Clinical observations: A careful clinical observation of each animal should be made at least once a day. Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, *e.g.*, necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals. All toxicological and pharmacological signs should be recorded including

time of onset, intensity, and duration. The time of death should also be noted. Individual records should be maintained for each animal.

5. Weight change: Animals must be weighed individually on the day the test substance is administered, weekly thereafter, and prior to sacrifice.

6. Necropsy: A complete gross necropsy should be performed on all animals that die during the course of the test. Consideration should be given to gross necropsy of the animals sacrificed at termination of the test. Microscopic examination of gross lesions should also be considered. If the substance will not be subjected to additional acute or multiple dose testing that includes gross necropsy, or if the results of this test are to be used for labeling purposes, complete gross necropsy should be performed on the remaining animals at termination of the test.

III. Data Reporting

A. Identification

Each test report should be signed by the persons responsible for the test and identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test including (a) the conduct of the test, (b) pathology, (c) analysis of the data, (d) the writing of the report, and (e) any written or other matter contained in the report.

B. Body of Report

The test report must include all information necessary to provide a complete and accurate description and evaluation of the test procedures and results, in the following sections:

1. Summary and conclusions: This section of the test report should contain a brief description of the methods, a summary of the data, an analysis of the data, and a statement of the conclusions drawn. The summary must highlight all positive data or observations and any deviations from control data which may be indicative of toxic effects.

2. Materials: This section of the test report should include, but not be limited to, the following information:

   (a) Identification of the test substance, so far as practical, including:

      i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities and listing the percentage of unidentifiable materials to account for 100% of the test sample.

      ii. manufacturer and lot number of the substance tested, and such information as physical state, pH, stability, and purity; and

      iii. specific identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

   (b) Animal data, including:

      i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

      ii. source of supply of the animals;

      iii. description of any pre-test conditioning;

iv. description of the method used in randomization of animals to test or control groups; and

v. numbers, age, and condition of animals of each sex in each test and control group.

(c) Data on husbandry should include description of the caging conditions including number of animals per cage, diet, bedding material, ambient temperature, humidity, and lighting conditions.

3. Methods:

(a) Deviation from guidelines - This section shall indicate all ways in which the test procedure deviates from these guidelines and shall state the rationale for such deviation.

(b) Specification of test methods - This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Statistical analysis - All statistical methods used should be fully described or identified by reference.

(d) Data on dosage administration, including:

i. all dose levels administered, expressed as mg/kg of body weight;

ii. method and frequency of administration; and

iii. total volume of substance (*i.e.*, test substance plus vehicle) contained in individual dosages.

(e) Data on observation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

4. Results:

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results.

(a) Tabulation of the response data (*i.e.*, number of animals dying; number of animals showing signs of toxicity; number of animals exposed) at each exposure level by sex, and time of death after dosing;

(b) LD_{50} values for each test substance calculated at the end of the obervation period, with method of calculation specified;

(c) 95% confidence interval for the LD_{50} values;

(d) Slope of the dose-mortality curve for each substance tested; and

(e) Findings from all clinical observations, necropsy, and histopathological examinations.

IV. Suggested Reading

1. Balazs, T. 1970. Measurement of Acute Toxicity. *In*: Methods in Toxicology. G. E. Paget, ed. F. A. Davis Co., Philadelphia, Pa.

2. Hagan, E. C. 1959. Acute Toxicity. *In*: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States.

3. Bliss, C. I. 1938. The determination of the dosage mortality curve from small numbers. Quarterly Journal Pharm. Pharmacol. 11:192-216.

4. Litchfield, J. T., Jr. and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Therap. 96:99-115.

5. Thompson, W. R. 1947. Using of moving averages and interpolation to estimate median effective dose. Bacteriological Rev. 11:115-145.

TESTING STANDARDS AND GUIDELINES WORK GROUP
INTERAGENCY REGULATORY LIAISON GROUP

Chairman:

Dr. Victor Morgenroth, III	Food and Drug Administration

Surrogate Liaison:

Dr. Allen H. Heim	Food and Drug Administration

Member:

Dr. James R. Beall	Department of Energy
Dr. William D'Aguanno	Food and Drug Administration
Dr. Charles Grieshaber	National Cancer Institute (NTP)
Dr. Stanley Gross	Environmental Protection Agency
Dr. Zdenka Horakova	Food Safety & Quality Service
Dr. Patricia Marlow	Occupational Safety & Health Administration
Dr. Joseph McLaughlin	Consumer Product Safety Commission
Dr. Robert Osterberg	Food and Drug Administration
Dr. Norbert Page	Environmental Protection Agency
Dr. Orville Paynter	Department of Commerce
Ms. Peggy Perry	Food and Drug Administration
Dr. John Quest	Food and Drug Administration
Mr. Van Seabaugh	Consumer Product Safety Commission
Dr. Frode Ulvedal	Environmental Protection Agency

The IRLG Testing Standards and Guidelines Work Group dedicates its guidelines to the memory of Dr. William D'Aguanno, whose commitment and talent contributed immeasurably to their development.